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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/077,572	10/13/1998	MICHAEL A. APICELLA	875001US2	6184
21186	7590	12/09/2003	EXAMINER	
SCHWEGMAN, LUNDBERG, WOESSNER & KLUTH, P.A. P.O. BOX 2938 MINNEAPOLIS, MN 55402			DEVI, SARVAMANGALA J N	
		ART UNIT	PAPER NUMBER	
		1645	43	
DATE MAILED: 12/09/2003				

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No. 09/077,572	Applicant(s) APICELLA ET AL.
	Examiner S. Devi, Ph.D.	Art Unit 1645

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1) Responsive to communication(s) filed on 08 September 2003.

2a) This action is **FINAL**. 2b) This action is non-final.

3) Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4) Claim(s) 22-26,29,32 and 33 is/are pending in the application.
4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5) Claim(s) _____ is/are allowed.

6) Claim(s) 22-26,29,32 and 33 is/are rejected. .

7) Claim(s) _____ is/are objected to.

8) Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9) The specification is objected to by the Examiner.

10) The drawing(s) filed on _____ is/are: a) accepted or b) objected to by the Examiner.

 Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

 Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11) The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. §§ 119 and 120

12) Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
a) All b) Some * c) None of:
1. Certified copies of the priority documents have been received.
2. Certified copies of the priority documents have been received in Application No. _____.
3. Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).
* See the attached detailed Office action for a list of the certified copies not received.

13) Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application) since a specific reference was included in the first sentence of the specification or in an Application Data Sheet. 37 CFR 1.78.
a) The translation of the foreign language provisional application has been received.

14) Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121 since a specific reference was included in the first sentence of the specification or in an Application Data Sheet. 37 CFR 1.78.

Attachment(s)

1) Notice of References Cited (PTO-892)
2) Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) Information Disclosure Statement(s) (PTO-1449) Paper No(s) 42.
4) Interview Summary (PTO-413) Paper No(s). _____.
5) Notice of Informal Patent Application (PTO-152)
6) Other: _____

RESPONSE TO APPLICANTS' AMENDMENT

Applicants' Amendment

- 1) Acknowledgment is made of Applicants' amendment filed 09/08/03 (paper no. 41) in response to the non-final Office Action mailed 04/03/03 (paper no. 39).

Status of Claims

- 2) Claims 22, 23, 25 and 29 have been amended via the amendment filed 09/08/03.
Claim 34 has been canceled via the amendment filed 09/08/03.
Claims 22-26, 29, 32 and 33 pending and are under examination.

Information Disclosure Statement

- 3) Acknowledgment is made of Applicants' Information Disclosure Statement filed 09/08/03 (paper no. 42). The information referred to therein has been considered and a signed copy of the same is attached to this Office Action (paper no. 43).

Sequence Listing

- 4) Acknowledgment is made of Applicants' submission of raw Sequence Listing and the CRF, which have been entered on 09/12/03.

Prior Citation of Title 35 Sections

- 5) The text of those sections of Title 35 U.S. Code not included in this action can be found in a prior Office Action.

Prior Citation of References

- 6) The references cited or used as prior art in support of one or more rejections in the instant Office Action and not included on an attached form PTO-892 or form PTO-1449 have been previously cited and made of record.

Priority - Specification

- 7) It is noted that a paragraph is introduced at the beginning of the specification on the first page providing priority information. The instant application is described as the national stage 371 application of PCT/US96/18994, filed 11/27/1996, which is a continuation-in-part application of the US application 08/565,943, filed 12/01/1995.

This amendment is objected to for not reciting the current co-pending status of the US application 08/565,943.

Objection(s) Maintained

8) The objection to the drawings made in paragraph 6 of the Office Action mailed 04/28/99 (paper no. 11) under 37 CFR 1.84 because of the reasons set forth by the Draftsperson is maintained for reasons set forth therein and in paragraph 7 of the Office Action mailed 04/03/03.

Objection(s) Withdrawn

9) The objection to the specification made in paragraph 9 Office Action mailed 04/03/03 (paper no. 39) with regard to sequence non-compliance is with Applicants' compliance with Sequence Rules.

Rejection(s) Moot

10) The rejection of claim 34 made in paragraph 11 of the Office Action mailed 04/03/03 (paper no. 39) under the judicially created provisional obviousness type double patenting over the cited claim(s) of the co-pending application, SN 09/565,943, is moot in light of Applicants' cancellation of the claim.

11) The rejection of claim 34 made in paragraph 12 of the Office Action mailed 04/03/03 (paper no. 39) under 35 U.S.C. § 112, first paragraph, as being non-enabled, with regard to the deposit issue, is moot in light of Applicants' cancellation of the claim.

12) The rejection of claim 34 made in paragraph 13 of the Office Action mailed 04/03/03 (paper no. 39) under 35 U.S.C. § 112, first paragraph, as containing new matter, is moot in light of Applicants' cancellation of the claim.

13) The rejection of claim 34 made in paragraph 14 of the Office Action mailed 04/03/03 (paper no. 39) under 35 U.S.C. § 112, first paragraph, as not providing adequate written description, is moot in light of Applicants' cancellation of the claim.

14) The rejection of claim 34 made in paragraph 15 of the Office Action mailed 04/03/03 (paper no. 39) under 35 U.S.C. § 112, second paragraph, as being indefinite, is moot in light of Applicants' cancellation of the claim.

Rejection(s) Withdrawn

15) The rejection of claims 22-26, 29, 32 and 33 made in paragraph 10 of the Office Action mailed 04/28/99 (paper no. 11), paragraph 24 of the Office Action mailed 10/11/00 (paper no. 25) and maintained in paragraphs 6 and 8 of the Office Action mailed 02/21/01 (paper no. 27) and

paragraph 12 of the Office Action mailed 04/03/03 (paper no. 39) under 35 U.S.C. § 112, first paragraph, is withdrawn

16) The rejection of claim 29 made in paragraph 15(a) of the Office Action mailed 04/03/03 (paper no. 39) under 35 U.S.C. § 112, second paragraph, as being indefinite, is withdrawn in light of Applicants' amendment to the claim.

17) The rejection of claim 22 made in paragraph 15(b) of the Office Action mailed 04/03/03 (paper no. 39) under 35 U.S.C. § 112, second paragraph, as being indefinite, is withdrawn in light of Applicants' amendment to the claim.

18) The rejection of claims 23 and 25 made in paragraph 15(d) of the Office Action mailed 04/03/03 (paper no. 39) under 35 U.S.C. § 112, second paragraph, as being indefinite, is withdrawn in light of Applicants' amendment to the claims.

19) The rejection of claims 22-26, 29, 32 and 33 made in paragraph 13 of the Office Action mailed 04/03/03 (paper no. 39) under 35 U.S.C. § 112, first paragraph, as containing new matter, is withdrawn in light of Applicants' amendment to the claims.

20) The rejection of claims 22-26, 29, 32 and 33 made in paragraph 14 of the Office Action mailed 04/03/03 (paper no. 39) under 35 U.S.C. § 112, first paragraph, as not having possession of the invention, is withdrawn.

21) The rejection of claims 29 and 33 made in paragraphs 15(c) and 15(e) of the Office Action mailed 04/03/03 (paper no. 39) under 35 U.S.C. § 112, second paragraph, as being indefinite, is withdrawn. A modified rejection is made below.

Rejection(s) Maintained

22) The rejection of claims 22, 23, 25 and 29 made in paragraph 9 of the Office Action mailed 04/28/99 (paper no. 11) and the rejection of claim 32 made in paragraph 23 of the Office Action mailed 10/11/00 (paper no. 25) and maintained in paragraph 11 of the Office Action mailed 04/03/03 (paper no. 39) under the judicially created provisional obviousness type double patenting over the cited claim(s) of application SN 09/565,943 is maintained for reasons set forth therein. Applicants have previously stated that they would consider filing a terminal disclaimer upon notification of allowable subject matter.

Rejection(s) under 35 U.S.C. § 112, Second Paragraph

23) Claims 22-26, 29, 32 and 33 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention.

(a) Claim 22 is indefinite and confusing in the recitation: ‘method of making a mutant endotoxin wherein the wild type pathogen produces wild type endotoxin, wherein mutating the *htrB* gene provides a mutant gram-negative bacterial pathogen that produces the mutant endotoxin’, because it is unclear how a method of making a ‘mutant endotoxin’ can include the production of both a wild type endotoxin and the production of a mutant endotoxin, as recited currently.

(b) Claim 22 is vague, confusing and/or grammatically incorrect in the recitation: ‘same as the lipid A of the wild type endotoxin except the lipid A’. The limitation is further inconsistent with the similar, but correct limitation used in part (b) of claim 29.

(c) Claim 22 is indefinite and/or has improper antecedence in the recitation: ‘the wild type pathogen’, because while there is an earlier recitation in the claim of ‘a wild type gram-negative bacterial pathogen’, there is no earlier recitation of any broader ‘wild type pathogen’.

(d) Claim 23 is indefinite, confusing and/or has improper antecedence in the recitation: ‘the gram-negative bacterial mutant pathogen’. Claim 23 depends from claim 22, which recites a ‘mutant gram-negative bacterial pathogen’, but not a ‘gram-negative bacterial mutant pathogen’.

(e) Claim 29 is confusing. The claim is drawn to a method of producing ‘endotoxin-specific antisera’. However, what is being collected in step (b) is not antisera, but “antibody specific for the *htrB* mutant produced from the immunized animal”. The ‘endotoxin-specific antisera’ is different in scope and specificity from ‘antibody specific for the *htrB* mutant’. What is being collected in step (b) of the claim is not what is being recited in the first line of the claim as being produced. An individual immunized, for example, with a *htrB* bacterial mutant would induce antibodies to different antigens present on the bacterial mutant. Furthermore, the recitation ‘the *htrB* mutant’ in part (b) is broadening in scope compared to the earlier recitation in the claim: ‘the *htrB* mutant of a gram-negative bacterial pathogen’. What element is included in the recitation: ‘the *htrB* mutant’ in part (b) of the claim is not clear, and therefore the specificity of the antibody collected in step b) of the claim is indeterminate.

(f) Claim 29 is vague and confusing because the claimed method is for producing

‘endotoxin-specific antisera’, but the product being used to immunize an individual is an *htrB* mutant of a gram-negative bacterial pathogen; or endotoxin isolated or purified from such a mutant (i.e., *htrB* endotoxin), and the product collected in step (b) is an ‘antibody specific for the *htrB* mutant’. Is the antisera recited in line 1 of the claim specific to wild-type endotoxin or *htrB* endotoxin? Is the antibody collected in step (b) specific for the *htrB* mutant bacterial pathogen, or the *htrB* mutant endotoxin?

(g) Claims 23-26, 32 and 33, which depend directly or indirectly, from claim 22 or claim 29 are also rejected as being indefinite because of the vagueness or indefiniteness identified above in the base claim.

Rejection(s) under 35 U.S.C § 112, First Paragraph (New Matter)

24) Claims 22-26 and 32 are rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. This is a new matter rejection.

Independent claim 22, as amended, now includes the limitation: ‘wherein the wild type pathogen produces wild type endotoxin, wherein mutating the *htrB* gene provides a mutant gram-negative bacterial pathogen that produces’ the mutant endotoxin. However, there appears to be no support in the specification for a method of making a mutant endotoxin which includes both the production of a wild type endotoxin and the production of a mutant endotoxin, as recited currently. Applicants have not pointed to a part of the specification that provides descriptive support for the currently added limitations. Therefore, the limitations in the claims are considered to be new matter. *In re Rasmussen*, 650 F2d 1212 (CCPA, 1981). New matter includes not only the addition of wholly unsupported subject matter but also, adding specific percentages or compounds after a broader original disclosure, or even omission of a step from a method. See M.P.E.P 608.04 to 608.04(c).

Applicants are respectfully requested to remove the new matter from the claim(s), or are invited to point to specific pages and line numbers in the originally filed specification where support for such recitations can be found.

25) Claims 22-26, 29, 32 and 33 are rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably

convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. This is a new matter rejection.

Independent claims 22 and 29, as amended, now include the limitation: 'lipid A of the' mutant endotoxin is the same as 'the lipid A of the' wild type endotoxin except the lipid A of the mutant endotoxin lacks one or more secondary acyl chains 'as compared to the' lipid A of 'the wild type endotoxin'. Applicants point to Figure 1 depicting hexaacyl wild type endotoxin; and Figures 2A and 2B depicting pentaacyl and tetraacyl lipid A from mutant endotoxins; Brief Description of the Figures on page 5 of the specification; and page 4, lines 18-25; page 11, lines 16-23 and page 18, lines 5-8 of the specification, as providing support for the new limitations. Applicants submit that the only change between Figure 1 and Figures 2A/2B is a decrease in the number of secondary acyl chains in the lipid A and that there is no other change, such as, length of the remaining chains in the lipid A. However, specific parts of the specification, as originally filed, do not appear to provide support for the claims, as amended currently. The evidence that there is new matter in the claims, as amended, can be found within the instant specification, and can be confirmed by the Gibson-Apicella Declaration filed along with Applicants' amendment filed 07/12/00 (paper no. 19); and the general structure depicted in Figures 1, 2A/2B in comparison with the actual structure of lipid A as known in the art; Figure 7; and the parts of the specification that describe the important features of Figure 7.

(A) The specification as originally filed, described that *htrB* mutation does affect phosphorylation of LOS (see lines 1 and 2 on page 17 of the specification).

(B) The paragraph 6 of the Gibson-Apicella Declaration filed along with Applicants' amendment filed 07/12/00 (paper no. 19) is reproduced herebelow, which confirms the disclosure in the specification with regard to the changes in the phosphorylation pattern particularly in the lipid A:

6. In addition, some **changes in the phosphorylation pattern in the LOS and lipid A moiety** are observed between wild type and *htrB*-mutant in *N. gonorrhoeae* strain 1291. These **changes involve an increased level of phosphoethanolamine (PEA) in both the lipid A moiety** as well as the oligosaccharide. [Emphasis added].

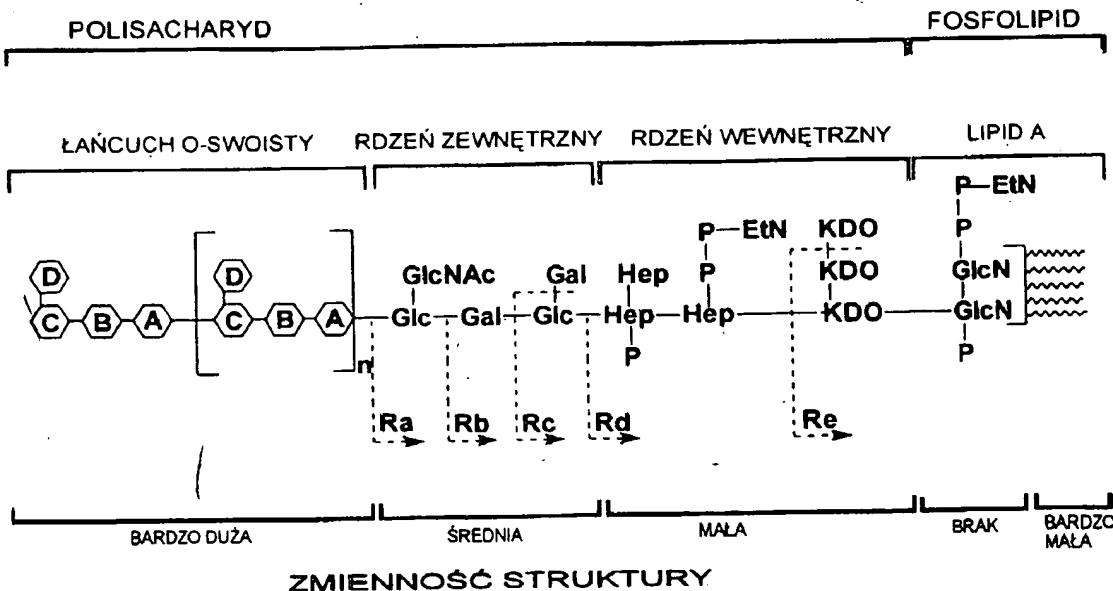
(C) Applicants state that 'Figure 1 depicts a wild type endotoxin (hexaacyl), and Figures 2A and 2B depict mutant endotoxins of the invention (pentaacyl and tetraacyl, respectively)'. Applicants also point to page 4 of the specification apparently describing Brief Description of the Figures, and state that the only changes between Figure 1 and Figures 2A/2B is a decrease in the

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number of secondary acyl chains. Applicants also point to page 4, lines 3-9; page 7, lines 7-10; and page 13, lines 1-5 of the specification. However, Figure 1, and Figures 2A and 2B appear to be limited to a general schematic representation of the lipid A of a Gram negative bacterium, before and after *htrB* mutation respectively. The 'Brief Description of the Figures' on page 4 of the specification describes that Figures 1, 2A and 2B represent 'a schematic representation of the general structure of lipid A' from a Gram negative enterobacterium and the LOS of an *htrB* mutant bacterium respectively. These Figures however do not appear to represent the complete structure of the lipid A moiety of a Gram negative bacterial endotoxin as known in the art and therefore, cannot depict the *changes* in the level of phosphoethanolamine (PEA) resulting from the *htrB* mutation, as stated above in the Gibson-Apicella Declaration. Consistent with the statement made in the Gibson-Apicella Declaration, the complete structure of the lipid A as recognized in the art includes phosphoethanolamine (PEA). The complete lipid A structure from three different publications are reproduced herebelow, all of which show that phosphoethanolamine (PEA) is a part of the lipid A moiety of a Gram negative bacterial endotoxin.

I). Jachymek W. *Post. Hih. Med. Dosw.* 49: 1: 171-178, 1995:



Ra - Re, chemotypy oligocukrów rdzeniowych

Ryc. 3. Schemat budowy lipopolisacharydu

II). Lipid A of *E. coli* and *Salmonella typhimurium* (Raetz CRH. *Annu. Rev. Biochem.* 59: 129-170, 1990):

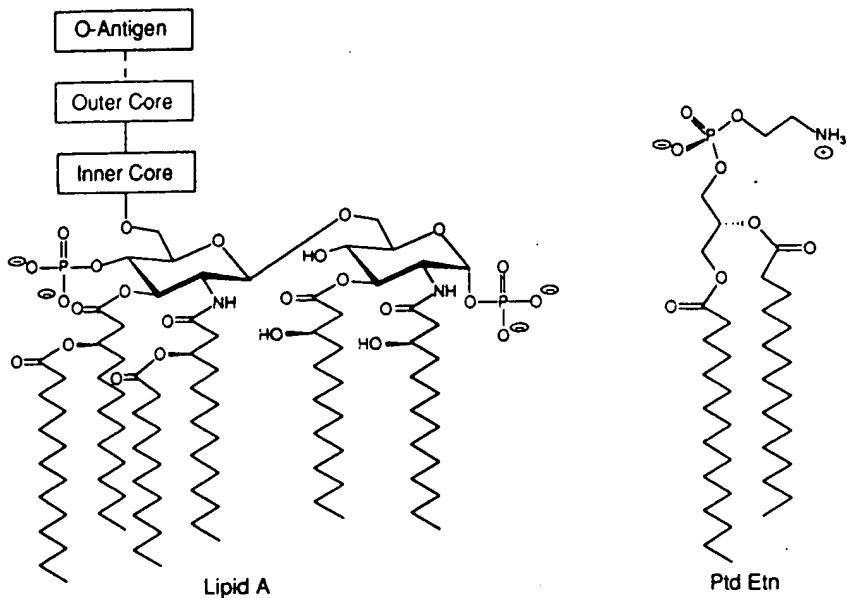


Figure 2 Covalent structure of *E. coli* and *S. typhimurium* lipid A. Phosphatidylethanolamine, the major glycerophospholipid (6) of these cells, is drawn to scale.

III). Lipid A of *Neisseria meningitidis* (Kulshin et al. *J. Bacteriol.* 174: 1793-1800, 1992):

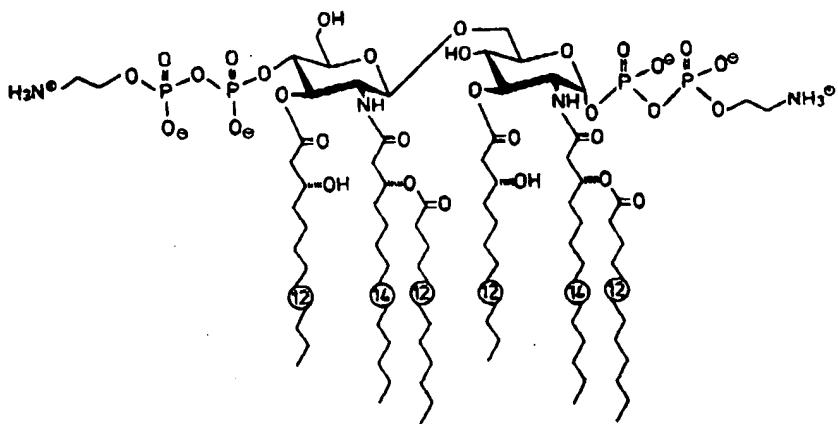


FIG. 2. Primary structure of the predominant hexaacyl lipid A component of *N. meningitidis* LPS. In lipid A, both the nonglycosidic (4'-P) and glycosidic (1- α -P) phosphate groups are substituted nonstoichiometrically but, to a large extent (80 to 85%), by Etn-P. The reducing GlcN I residue is assumed to be present in the α -anomeric form, and the 3-hydroxy fatty acids possess the R configuration. The hydroxyl group at C-6' represents the attachment site of the oligosaccharide portion. The numbers in circles show the number of atoms in acyl chains.

In the instant application, Figure 1 does not appear to depict the presence of Etn-P in the wild-type lipid A. Figures 2A and 2B do not depict the presence or absence of phosphoethanolamine (PEA) or Etn-P in the lipid A moiety of the *htrB* mutant endotoxin, and therefore, these Figures do not and cannot represent the *changes* in the level of PEA within the lipid A moiety that occur as a result of the *htrB* mutation. Therefore, these Figures cannot provide descriptive support for the limitations identified above in the claims, as amended.

(D) More importantly, Figure 7 of the instant specification; the 'Brief Description' for Figure 7 on page 6; and the description in the paragraph bridging pages 48 and 49 of the specification describing the structures of wild type lipid A and *htrB* mutant lipid A - all in combination provide the *prima facie* evidence that the lipid A moiety of an endotoxin of a *htrB* mutant of a Gram negative bacterial pathogen is **not** the same as the lipid A of the wild type endotoxin except for the lack of one or more secondary acyl chains, as recited currently. Figure 7 is the schematic representation of the wild type *Salmonella* lipid A and the lipid A of the *htrB* mutant *Salmonella* (see top of page 6 of the specification). This Figure 7 and the last paragraph on page 48 of the specification describe that *htrB* mutation results in substitution of the C12 fatty acid with C16 fatty acid on the N linked C14 fatty acid. For example, the paragraph bridging pages 48 and 49 of the instant specification states as follows:

The chemical analysis of the lipid A of the *S. typhimurium* *htrB* mutant indicated that the modifications in the lipid A structure that occurred were ... **not identical** to modifications in the lipid A structure seen in *H. influenzae* *htrB* mutants. In the wild type strain, on glucosamine II, the 3' substitution on the N-linked C14 fatty acid (hexaacyl or heptaacyl) is a C12 fatty acid. In contrast, and as shown on FIG. 7, the **C12 fatty acid is replaced** with a C16 fatty acid. Mutation of the *S. typhimurium* *htrB* gene results in the functional induction of another acyltransferase which places a C16 fatty acid at the 3' position on the N-linked C14 fatty acid. [Emphasis added].

Therefore, the limitation: 'wherein the lipid A of the mutant endotoxin is the same as the lipid A of the wild type endotoxin except .. the lipid A of the mutant endotoxin lacks one or more secondary acyl chains as compared to the lipid A of the wild type endotoxin', clearly constitutes new matter. *In re Rasmussen*, 650 F2d 1212 (CCPA, 1981). New matter includes not only the addition of wholly unsupported subject matter but also, adding specific percentages or compounds after a broader original disclosure, or even omission of a step from a method. See M.P.E.P 608.04 to 608.04(c).

Applicants are respectfully requested to remove the new matter from the claim(s), or are

invited to point to specific pages and line numbers in the originally filed specification where support for such recitations can be found.

Rejection(s) under 35 U.S.C. § 112, First Paragraph (Scope of Enablement)

26) Claims 22-26, 29, 32 and 33 are rejected under 35 U.S.C. 112, first paragraph, because the specification while being enabling for: (A) a method of making a mutant endotoxin from a non-typeable *Haemophilus influenzae* strain having a mutation in the *htrB* gene, wherein the *htrB* mutation produces a mutant endotoxin containing a decreased phosphoethanolamine content and an increased hexose content in the inner core of said mutant endotoxin, and a pentaacylated or tetraacylated lipid A lacking one or both of the two secondary acyl chains compared to the hexaacylated endotoxin of a wild type non-typeable *Haemophilus influenzae*, and wherein the mutant endotoxin has substantially reduced toxicity when compared to the wild-type endotoxin; and purifying the mutant endotoxin from the *htrB* mutated non-typeable *Haemophilus influenzae*; (B) the mutant endotoxin made according to the method; and (C) a method of producing antisera containing antibodies specific to the *htrB* mutant endotoxin, does not reasonably provide enablement for: (i) a method of making a mutant endotoxin comprising mutating an *htrB* gene in any wild type gram-negative bacterial pathogen, wherein the wild type pathogen produces wild type endotoxin, wherein mutating the *htrB* gene provides a mutant gram-negative bacterial pathogen that produces the mutant endotoxin, wherein the lipid A of the mutant endotoxin is the same as the lipid A of the wild type endotoxin except that the lipid A of the mutant endotoxin lacks one or more secondary acyl chains as compared to the lipid A of the wild type endotoxin, and wherein the mutant endotoxin has substantially reduced toxicity when compared to the wild type endotoxin, and purifying the mutant endotoxin from the mutant gram-negative bacterial pathogen, as claimed in a broad sense; (ii) the mutant endotoxin made by said method; and (iii) a method for producing endotoxin-specific antisera, as claimed broadly.

Instant claims are evaluated based on the *Wands* analysis. Many of the factors regarding undue experimentation have been summarized in *In re Wands*, 858 F.2d 731, 8 USPQ2d 1400 (Fed. Circ. 1988) as follows:

- The quantity of experimentation necessary (time and expense);
- The amount of direction or guidance presented;
- The presence or absence of working examples of the invention;

- The nature of the invention;
- The state of the art;
- The relative skill of those in the art;
- The predictability or unpredictability of the art; and
- The breadth of the claims.

In the instant case, the nature of the invention includes mutating the *htrB* gene in a wild-type Gram negative bacterial pathogen which would result in a mutant endotoxin that is recited to contain a lipid A ‘wherein the lipid A of the mutant endotoxin is the same as the lipid A of the wild type endotoxin except that the lipid A of the mutant endotoxin lacks one or more secondary acyl chains as compared to the lipid A of the wild type endotoxin’, wherein the mutant endotoxin has substantially reduced toxicity when compared to the wild type endotoxin. A method of making such a mutant endotoxin; the mutant endotoxin made by the method; and a method of producing antisera specific to an endotoxin are claimed. Although the relative skill of those in this art is high, the breadth of the claims encompasses making such a mutant endotoxin from any wild type Gram negative bacterial pathogen; a method of making such a mutant endotoxin; and an antiserum to such a product. The term ‘wild type Gram-negative bacterial pathogen’ includes a myriad of Gram negative bacterial pathogens including Gram negative cocci and bacilli; and aerobic or anaerobic Gram negative bacterial pathogens belonging to different bacterial families. The specification mentions of a list of Gram negative bacterial pathogens as being included in the scope of the invention: *E. coli*; *H. influenzae*; *P. aeruginosa*; *P. pseudomallei*; *P. mallei*; *S. typhimurium*; *S. typhi*; *S. paratyphi*; *S. newport*; *S. enteritidis*; *S. newport*; *N. meningitidis*; *N. gonorrhoeae*; *H. ducreyi*; *Campylobacter jejuni*; *Moraxella catarrhalis*; *Shigella dysenteriae*; *S. sonnei*; and *S. flexneri* (see pages 12 and 24; and Example 6-12). Claims 32 and 33 encompass any species of the genera *Haemophilus*, *Neisseria*, *Moraxella*, *Campylobacter*, *Shigella* and *Pseudomonas*. However, there is no enabling disclosure showing that a mutation in *htrB* gene, if indeed such a gene exists in all these Gram negative bacteria, would result in a mutant endotoxin containing a lipid A ‘wherein the lipid A of the mutant endotoxin is the same as the lipid A of the wild type endotoxin except that the lipid A of the mutant endotoxin lacks one or more secondary acyl chains as compared to the lipid A of the wild type endotoxin’. Except for a strain of non-typeable *Haemophilus influenzae*, *E. coli* and *S. typhimurium*, the presence of a *htrB* gene in all the above-mentioned Gram negative bacterial pathogens was neither known at the time of the invention, nor is it shown to exist, within the instant specification as filed, in

these bacterial pathogens. The Examples speculate that there 'may be' sufficient homology in *htrB* gene between *H. influenzae* and all the rest of the Gram negative bacteria cited above. However, such a mere speculative statement that the invention or the method 'may' work within the broad genus of Gram negative bacterial pathogens, without a concrete showing, is insufficient to meet the scope of enablement requirement of the claimed invention. The specification fails to teach mutant endotoxin species having a lipid A with the specific structure as recited, from a representative number of species of Gram negative bacterial pathogens, sufficient to allow one skilled in the art to determine that the entire scope of the instant claims is indeed enabled. This is critical in light of the predictability or unpredictability factor which forms a part of *Wands* analysis. A review of the instant disclosure and the state of the art at the time suggests that the entire scope of the claims is not enabled, particularly in light of the unpredictability involved in obtaining, from a representative number of species of Gram negative bacterial pathogens, an *htrB* mutant endotoxin having a lipid A that is the same as the lipid A of the wild type endotoxin except for the lack of one or more secondary acyl chains as compared to the lipid A of the wild type endotoxin. Applicants state that they need not demonstrate the efficacy of all species of gram negative pathogens in order to be entitled to a generic claim of reasonable scope. Applicants submit that the lipid A moiety of the endotoxin is highly conserved among bacteria of the family *Enterobacteriaceae* and closely related gram-negative bacteria, and therefore one of ordinary skill in the art would expect that any gram-negative bacterial pathogen would be capable of producing a mutant endotoxin of substantially reduced toxicity as compared to the endotoxin of a wild type bacterial pathogen of the same species. Applicants assert that the present application presents a detailed description of how to make the claimed invention using standard mutagenesis techniques and that one of skill in the art can use standard screening methods, such as, mass spectrometry. Applicants point to the illustrative examples for *Haemophilus* in Examples 1-3 of the specification. Applicants submit that paragraph 5 of the Gibson-Apicella Declaration provides evidence that the outcome for a knockout *htrB* gene in *N. gonorrhoeae* is similar to the outcome for an *htrB* knockout gene in *H. influenzae*. However, the disclosure and the evidence of record points to the contrary. Although lipid A portions of endotoxins of some Gram negative bacterial pathogens may be conserved, they are not identical. Applicants state that the first paragraph of 35 U.S.C. § 112, first paragraph, does not require literal

support for the claimed invention. Applicants submit that one with ordinary skill in the art upon reading the full specification would understand that the claimed mutant endotoxin is the same as wild type endotoxin except for lacking one or more secondary acyl chains of lipid A. However, the disclosure and the evidence of record points to the contrary. Applicants have not shown that mutation in the *htrB* gene of one Gram negative bacterial pathogen as claimed would automatically predict the production of a mutant endotoxin having a lipid A that is the same as the wild type lipid A except for the lack of one or more secondary acyl chains, in another Gram negative bacterial pathogen. The instant specification itself provides the *prima facie* evidence that changes in the lipid A component of one Gram negative bacterial endotoxin due to *htrB* mutation cannot be predicted to occur in the lipid A moiety of another Gram negative bacterial endotoxin. The following analysis is made using the disclosure within the instant specification; the state of the art at the time of the invention; the evidence provided via the Gibson-Apicella Declaration; and the disclosure from a post-filing reference.

The instant specification demonstrates that one *htrB* endotoxin mutant species obtained from one Gram negative bacterial species, a non-typeable *H. influenzae* 2019, has a mixture of pentaacyl and tetraacyl lipid A lacking one or two secondary acyl or myristoyl groups; shows a decreased phosphoethanolamine content and an increased hexose content in the inner core of the mutant endotoxin. See the last paragraph on page 16 of the specification. Lines 1 and 2 of page 17 of the instant specification describe that *htrB* mutation does affect phosphorylation of LOS. The prior art at the time of the invention on the other hand, documented variable effect(s) brought about by *htrB* mutation in different Gram negative bacteria. For instance, Lee *et al.* (*J. Biol. Chem.* 270: 27151-27159, 1995 - already of record) taught the changes in molecular ions effected in the lipid A moiety by the *htrB* mutation in a Gram negative bacterial pathogen, such as *Haemophilus*. Lee's Figure 7 showed changes different from the one described by Applicants in that there were changes seen at m/z 1725 and 1727 of the ion LSIMS spectra of the lipid A, indicating the loss of HPO₄ and H₃PO₄ in the lipid A. With yet another Gram negative bacterial pathogen, *E. coli*, Karow *et al.* (*J. Bacteriol.* 174: 7407-7418, 1992 - already of record) obtained a mutant endotoxin following a *htrB* mutation which contained a lipid A that differed from the lipid A of the instant invention in at least two different ways. The Gibson-Apicella Declaration at paragraph 8 acknowledges this variability in

stating that these changes represent the addition of new C16 fatty acids in place of the C12 fatty acid, and therefore represent a new hexaacylated lipid A molecule resulting from *htrB* mutation.

Further *prima facie* evidence that the claimed method of making a mutant endotoxin, the endotoxin made, and the method of producing antisera thereto, are not extrapolatable to any Gram negative bacterial pathogen as recited generically, comes from within the instant specification. The instant specification demonstrates that a mutation in *htrB* gene similar to the one effected in a non-typeable *H. influenzae* strain, when carried out in a heterologous Gram negative bacterial pathogen species, for example, *Salmonella typhimurium*, did **not** result in a mutant endotoxin wherein the lipid A remained the same as the lipid A of the wild-type endotoxin except for the lack of one or more secondary acyl chains, as recited currently. For instance, the paragraph bridging pages 48 and 49 of the specification specifically states as follows:

The chemical analysis of the lipid A of the *S. typhimurium* *htrB* mutant indicated that the modifications in the lipid A structure that occurred were ... **not identical** to modifications in the lipid A structure seen in *H. influenzae* *htrB* mutants. In the wild type strain, on glucosamine II, the 3' substitution on the N-linked C14 fatty acid (hexaacyl or heptaacyl) is a C12 fatty acid. In contrast, and as shown on FIG. 7, the **C12 fatty acid is replaced** with a C16 fatty acid. Mutation of the *S. typhimurium* *htrB* gene results in the functional induction of another acyltransferase which places a C16 fatty acid at the 3' position on the N-linked C14 fatty acid. [Emphasis added].

Figure 7 of the specification depicts such changes which are clearly different from the changes depicted via Figures 2A and 2B. This demonstrates that the *S. typhimurium* *htrB* mutant produces a mutant endotoxin wherein the changes made to the lipid A are non-identical to the changes that occur in the lipid A moiety of an *htrB* mutant of another Gram negative bacterial pathogen, such as, a non-typeable *H. influenzae*, and involves the replacement of the C12 fatty acid with a C16 fatty acid at the 3' position on the N-linked C14 fatty acid. Although a method analogous to the one applied for NT *H. influenzae* is described as being used in *S. typhimurium* (see lines 22 and 23 on page 46 of the specification), the resultant *S. typhimurium* *htrB* mutant LPS has a C16 fatty acid rather than C12 fatty acid on the N linked C14 fatty acid (see Figure 7; and last paragraph on page 48 of the specification). The heptaacyl or hexaacyl lipid A of the *htrB* mutant *Salmonella* had the same number of secondary acyl chains as the wild type lipid A. Such an unpredictability is well appreciated in the art as described in a post-filing reference. For instance, see last paragraph in column 2; and the first two paragraphs in column 3 of the US patent 6,482,807, published in

November 2002, which recognize the difficulty one skilled in the art would have in predicting the same changes that were produced in the lipid A of NT *H. influenzae* in the lipid A of any Gram negative bacterial pathogen, for example, in the lipid A of *Neisseria meningitidis*, *Moraxella*, *Campylobacter* etc. This patent states that while the structure of the lipid A of *Neisseria meningitidis* has been analyzed in the art, 'nothing is known concerning genetic make up of Neisseria with regard to presence or absence of a *htrB* gene or identity thereof' and that 'nothing is known of the influence any mutation in such a gene if it could be found would have on the resulting mutant strain or on the resulting product or products' (see first and fourth paragraphs in column 3 of the US patent 6,482,807). In light of this Applicant-demonstrated and the art-recognized unpredictability in obtaining a mutant endotoxin having a lipid A that is the same as the wild type lipid A except for the lack of one or more secondary acyl chains, one of skill in the art would not be able to extrapolate Applicants' experiments and results obtained with a *htrB* mutated non-typeable *H. influenzae* to any other generic Gram negative bacterial pathogen, without undue experimentation.

Furthermore, the documents that are made of record within the instant application confirm or provide additional evidence to support what is demonstrated in the instant specification and what is documented in the post-filing art. For instance, the Gibson-Apicella Declaration at paragraph 7 states that an art-known *htrB* mutation in another Gram negative bacterium, *E. coli* (the Karow mutant), resulted in a lipid A structure that is 'different in two very important ways from the *htrB* mutant pathogens of the present invention'. The Gibson-Apicella Declaration at paragraph 6 additionally describes further changes in the phosphorylation pattern of the lipid A moiety occurring in the *htrB* mutant of another Gram negative bacterial pathogen, a *Neisseria* species, which changes involve an increased level of phosphoethanolamine (PEA) in the lipid A portion. Thus, the evidence within the instant specification; the information submitted via the Gibson-Apicella Declaration; and the Lee *et al.* and Karow *et al.* publications; and the post-filing evidence disclosed by the US patent 6,482,807, all together establish that the instant claims are not enabled over the whole breadth. In view of the lack of adequate disclosure and guidance, the unfounded breadth of the claims, and the unpredictability factor established within the specification as well as in the relevant art, and the quantity of experimentation necessary, undue experimentation would have been required to reproducibly practice the full scope of the invention, as claimed. The instant claims are viewed as

not meeting the scope of enablement provision of 35 U.S.C. § 112, first paragraph.

The courts have held that it is the specification, not the knowledge of one skilled in the art, that must supply the novel aspects of an invention in order to constitute adequate enablement. See *Genentech Inc., v. Novo Nordisk A/S Ltd.*, 42 USPQd 1001. Moreover, the specification must have been enabling at the time the invention was made and developments after the time of filing are of no consequence to what one skilled in the art would have believed at the time of filing (*In re Wright*, 27 USPQ2d 1510, *Federal Circuit*, 1993).

Relevant Prior Art

27) The prior art made of record and not relied upon currently in any rejection is considered pertinent to Applicants' disclosure:

- Powell *et al.* (US 6,548,287, filed 11/22/1995) disclosed non-pyrogenic *htrB* mutants of Gram negative bacteria; substantially pure non-pyrogenic lipid A and vaccines comprising the same (see abstract; claims; and Examples).

Remarks

28) Claims 22-26, 29, 32 and 33 stand rejected.

29) Papers related to this application may be submitted to Group 1600, AU 1641 by facsimile transmission. Papers should be transmitted via the PTO Fax Center located in Crystal Mall 1. The transmission of such papers by facsimile must conform with the notice published in the Official Gazette, 1096 OG 30, November 15, 1989. The CM1 facsimile center's telephone number is (703) 308-4242. The RightFax number for submission of before-final amendments is (703) 872-9306. The RightFax number for submission of after-final amendments is (703) 872-9307.

30) Any inquiry concerning this communication or earlier communication(s) from the Examiner should be directed to S. Devi, Ph.D., whose telephone number is (703) 308-9347. A message may be left on the Examiner's voice mail service. The Examiner can normally be reached on Monday to Friday from 7.15 a.m to 4.15 p.m. except one day each bi-week which would be disclosed on the Examiner's voice mail system.

If attempts to reach the Examiner by telephone are unsuccessful, the Examiner's supervisor, Lynette Smith, can be reached on (703) 308-3909.

S. Devi, Ph.D.
S. DEVI, PH.D.
PRIMARY EXAMINER